

Study on the Anti-Aging Physiological Characteristics and Molecular Mechanism of *Camellia Oleifera*

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Research Article

Keywords: *Camellia oleifera*, transduction factor, exuberant vitality, sequencing analysis technique

Posted Date: May 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-493148/v1>

Abstract

To study the physiological and molecular regulating mechanism of ancient *Camellia oleifera* which kept a exuberant vitality for more than one hundred years, leaves of 30a year old and > 100 year old *Camellia oleifera* were selected as targets. On the basis of the study of the generation and the clearance of reactive oxygen species, sequencing analysis of the transcriptome and expression profiling by high throughput sequencing analysis technique was conducted to study differentially expressed functional genes related to the tree age. It showed that the chlorophyll content and enzyme activities increased in ancient *Camellia oleifera* leaves. Expression of chlorophyll a/b binding protein gene, auxin related gene, the signal transduction factor and the transcription factor gene in ancient trees were all higher than mature tree. The down regulated gene expression of inductive genes related to protein degradation in ancient tree. Under the comprehensive function of those factors, ancient *Camellia oleifera* leaves still kept an exuberant vitality which was very useful for studies of stress resistance molecular biology and genetic improvement of *Camellia oleifera*.

Introduction

Tree senescence refers to the process of decline occurring in plants or some organs during growth and development, which leads to the natural termination of life activities^{1,2}. Usually, during tree growth and development, the changes of internal factors, such as hormones, metabolic levels, aging-related genes, transcription factors and the effects of external environment factors, such as drought, high temperature, pests may lead to chlorophyll degradation, protein, lipids, nucleic acid, starch and other biological macromolecules hydrolysis^{3,4}, and cause, accelerate aging, and even cause tree death. At present, the research on the mechanism of tree senescence includes stomatal regulation theory, senescence gene regulation theory, active oxidation theory, nutrition deficit theory, plant hormone regulation theory, external stress and pest theory^{5,6}. The commonness of these theories is to acknowledge that plant senescence and death are the universal laws of life development, and are an active and necessary process in plant growth and development, morphogenesis and environmental response. Moreover, these theories also show that tree senescence is formed under the combined action of many different factors. At the same time, studies have found that different tree senescence processes are different due to different mechanisms and inducing factors of senescence⁷. When encountering many external stress and internal senescence related factors, some plants could form the corresponding resistance as well as the defense mechanism by some antioxidase, the transcription factor, and the signal transduction factor and thus prolonged its senescence process.

Camellia oleifera which were plants of *Camellia* family, *Camellia* plants and perennial small trees or shrubs was a specific oleiferous tree species of China which usually distributed at low mountains and hills in South of China⁸. *Elaeis guineensis*, *Olea europaea*, *Cocos nucleifera* as well as *Camellia oleifera* were four edible oleiferous tree species of the world. *Camellia oleifera* possessed characteristics of poor soil tolerance, strong resistance, and exuberant vitality. It was common to find the growth of *Camellia oleifera* with more than 100 year old age in natural forests. For example, there was a *Camellia oleifera* tree with more than 120 year old age grown in Hendong Country, Henyang City in China (N27°05', E112°56'). This single tree produced annual average *Camellia* fresh fruit for more than 200 kg during the continuous field observation from 2014 to 2016. What were the factors which maintained the exuberant vitality of those ancient *Camellia oleifera*? Nowadays, studies about the transcription level of plants antisenescence mainly focused on the model plants such as *Arabidopsis*

thaliana^{9,10}. However, research about antisenescence of *Camellia oleifera* was scarce. In this study, focusing on antisenescence of *Camellia oleifera*, leaves of *Camellia oleifera* with different ages were selected as targets. ROS and antioxidase activities in leaves of mature tree (30 a) and ancient tree (> 100 a) were determined. By conducting the transcriptome sequencing analysis, the ROS clearance system of *Camellia oleifera* with different ages as well as the expression of differential genes was studied. Furthermore, antisenescence physiological characteristics and molecular mechanism of ancient *Camellia oleifera* were studied preparing for the further studies of stress resistance molecular biology and genetic improvement.

Materials And Methods

Experimental materials

The experimental site was located in the experimental forest farm of Hunan Academy of Forestry (east longitude 113°01'20", north latitude 28°06'40"). On April 18th, 2017, good single plant of *Camellia oleifera* of mature tree (30 a) and ancient tree (> 100 a) with strong growth and good development were sampled (Fig. 1). Annual leaves which received consistent illumination, were strong and had no pests and diseases in those single plants at the middle and upper part of the periphery of crown were selected as experimental material, which had three biological replicates. The collected leaves were put into the plastic bags, numbered, quickly put into the ice box and taken back to the laboratory. After rinsing by deionized water and treatment with liquid nitrogen, leaves were put into - 70°C refrigerator for further analysis.

Determination methods

Contents of chlorophyll (Chl), malondialdehyde (MDA), hydrogen peroxide (H₂O₂), SOD, POD and CAT were determined by the extraction of acetone, the thiobarbituric acid method, the trichloroacetic acid colorimetry, the riboflavin-NBT photoreduction, the guaiacol method and the permanganate titration method, respectively.

Samples were sent to Beijing Genomics Institution in Guangzhou to conduct the transcriptome sequencing. The paired end sequencer of Illumina Hiseq TM 2000 was used for sequencing. Raw data were processed by Beijing Genomics Institution in Guangzhou.

Data analysis

Excel 2003 was used for the data processing and the figure drawing. SPSS 16.0 was used for data statistics and the *variance* analysis. The differential significance of the physiological and biochemical indexes was analyzed by one-way ANOVA test. Multiple comparisons used the least significant difference test (LSD).

Statement

We confirm that the use of plants in the present study complies with international, national and/or institutional guidelines.

Results

Antisenescence physiological indexes analysis of Camellia oleifera trees with different ages

There was a significant difference of chlorophyll content among *Camellia oleifera* leaves with different ages. Chl a + Chl b content in leaves of ancient *Camellia oleifera* was 0.44 mg/g, which was higher than 0.27 mg/g in mature tree. Ratio of Chl a to Chl b in leaves of ancient *Camellia oleifera* were higher than mature tree, which were 10.32 \pm 6.34, respectively. MDA and H₂O₂ contents in leaves of ancient *Camellia oleifera* were also higher than mature tree, which were 8.83 nmol/g and 126.77 μ g/g. Antioxidase activities of SOD, POD and CAT were the highest in ancient *Camellia oleifera* leaves, namely 671.00 (U/g), 22.58 U/(g min) and 498.42 U/(g min), respectively (Table 1). CAT was specific-functional enzyme for the clearance of H₂O₂. POD was also very important for the clearance of surplus H₂O₂ in plants. The changing trends of these two enzymes were negatively correlated with the change of H₂O₂ to some extent, illustrating that these two could promote the defense of *Camellia oleifera* against the attack of H₂O₂ and thus prolong senescence. SOD mainly cleared superoxide anion radical (O₂⁻).

In all, with the increase of the tree age, MDA and H₂O₂ contents of *Camellia oleifera* leaves increased, which resulted in excessive or unbalanced ROS in plants and final senescence. Meantime, *chlorophyll* in ancient *Camellia oleifera* leaves maintained at a relatively higher level. Besides, ancient *Camellia oleifera* leaves possessed relatively higher antioxidant activities, which not only effectively guaranteed photosynthesis but also defended the possibly adverse effect caused by the increase of MDA and H₂O₂ contents. Finally, the normal growth and development was promoted and the senescence process was prolonged.

Table 1 Chlorophyll content in *Camellia oleifera* leaves of different tree ages

	Chl a/Chl b	Chl a+ Chl b (mg/g)	MDA (nmol/g)	H ₂ O ₂ (μ g/g)	SOD (U/g)	POD(U/(g·min))	CAT(μ g/(g·min))
Ancient tree	10.32a(0.44)	0.44a (0.003)	8.83a (1.25)	126.77a (4.75)	671.00a (35.82)	22.58a (1.01)	498.42a (34.45)
Mature tree	6.34c(0.20)	0.27c (0.007)	6.51ab (0.63)	109.43b (1.99)	519.94a (17.56)	14.34ab (1.00)	363.80ab (19.40)

Note: Different letters illustrated the difference of physiological and biochemical indexes among different tree ages (P<0.05). Data in brackets were standard deviation.

The differential expression of antisenesescence related genes of *Camellia oleifera* leaves in stages of different tree ages

The differential expression of chlorophyll degradation related genes

Chlorophyll a/b binding protein gene was very important for the maintenance of the chlorophyll content *and* the photosynthetic ability of the plant. In this study, 10 differentially expressed related genes of chlorophyll a/b-binding protein were found in ancient *Camellia oleifera* leaves, wherein 9 genes were up regulated expressed and 1 gene was down regulated expressed (Table 2). The expression increase of Chlorophyll a/b binding protein gene possibly prolonged the senescence process by enhancing the photosynthetic ability of ancient *Camellia oleifera* tree.

Table 2 Differential expression of chlorophyll a/b-binding protein related genes

Gene category	Main functions	Differential gene	Up regulated gene amount(Ancient tree VS Mature tree)	Down regulated gene amount(Ancient tree VS Mature tree)
Chlorophyll a/b-binding protein	Maintain chlorophyll content and photosynthetic capacity of plants	10	9	1

The differential expression of antioxidant related genes

SOD, POD, CAT and APX were common antioxidants, which were usually very important for the clearance of surplus ROS in plants and the prolongation of senescence. In this study, 22 antioxidant genes were found significantly differentially expressed in ancient *Camellia oleifera* leaves, wherein 15 antioxidant genes were up regulated expressed and 7 genes were down regulated expressed (Table 3).

These antioxidant genes mainly included SOD, POD, CAT and APX. POD had the most differential genes amount, followed by those of APX, SOD and CAT. The increase of the expression of antioxidant genes could prolong the senescence process by enhancing the antioxidant activity of ancient *Camellia oleifera* trees.

Table 3 Differential expression of antioxidant related genes

Gene categories	Main functions	Differential gene amount	Up regulated gene amount(Ancient tree VS Mature tree)	Down regulated gene amount(Ancient tree VS Mature tree)
SOD	To clear surplus radicals	2	0	2
POD	To clear ROS and enhance the stress resistance to drought etc.	10	9	1
CAT	To clear surplus H ₂ O ₂	1	0	1
APX	To clear ROS	9	6	3
Total		22	15	7

The differential expression of hormone related genes

Hormone levels were closely related to plant senescence. Usually, Auxin and cytokinin (CTK) could prolong plant senescence, whereas abscisic acid (ABA) could accelerate plant senescence. In this study, dramatic differential expression of 40 hormone related genes were found in ancient *Camellia oleifera* leaves, wherein 21 genes were up regulated and 19 genes were down regulated expressed (Table 4). From categories, hormone related genes

mainly included ABA, auxin, CKX and SAUR, wherein auxin related differential gene number was the highest, which was followed by that of ABA. Due to the combined effects of those hormone related genes, ancient *Camellia oleifera* could maintain hormones at a relatively stable level and further its exuberant vitality.

Table 4 Differential expression of hormone related genes

Gene categories	Main functions	Differential gene amount	Up regulated gene amount(Ancient tree VS Mature tree)	Down regulated gene amount(Ancient tree VS Mature tree)
ABA	Inhibition of plant growth, promotion of leaf abscission and acceleration plant dormancy	12	6	6
Auxin	Promotion of the generation of lateral and adventitious root adjustment of flowering and sex differentiation, adjustment of fruiting and fruit development, and control of apical dominance	25	13	12
CKX	Maintenance or reestablishment of the stability and equilibrium of CTK in plants, enhancement of antioxidation ability of plants	1	1	0
SAUR	Maintenance of auxin level, adjustment of auxin transport and cell amplification	2	1	1
Total		40	21	19

The differential expression of stress resistance related genes

The transcription factor, the signal transduction factor, the resistance gene and the defense related gene could significantly enhance the resistance of plants and further effectively answer the external adverse growth factors, respectively. 496 stress resistance related genes were significantly differentially expressed in ancient *Camellia oleifera* leaves, wherein 301 genes were up regulated expressed and 195 genes were down regulated expressed. Those stress resistance genes mainly included transcription factors of MYB, NAC, MRKY and zinc Finger etc., signal transduction factors of GTP-binding, receptor protein kinase etc., resistance protein of pathogenesis related protein, disease resistance protein etc., and defense-related gene. The amount of differential genes related to Zinc Finger and disease resistance protein was the highest, followed by other genes of LRR receptor-like serine/threonine-proteinkinase, HSP, MYB, Leucine zipper, bHLH and ERF (Table 5). With the combined effects of those stress resistance related genes, ancient *Camellia oleifera* could maintain normal growth through the corresponding adjustment and adaption mechanism to response the external growth stress.

Table 5 The differential expression of stress resistance related genes

Gene categories	Main functions	Differential gene amount	Up regulated gene amount(Ancient tree VS Mature tree)	Down regulated gene amount(Ancient tree VS Mature tree)
Zinc Finger	Defense against drought, high temperature, salt stress, pathogen	128	73	55
HSP	Defense against high temperature and drought	39	24	15
MYB	Defense against drought, salt, coldness, high temperature stress and enhancement of POD activities of plants	30	15	15
bHLH	Clearance of ROS and further enhancement of coldness resistance of plants	31	22	9
Leucine zipper	Defense against drought and salt stress	21	18	3
WRKY	Defense against drought and salt stress	20	18	2
NAC	Defense against drought, high temperature, pathogenic bacteria	10	10	0
MADS-box	Defense against drought and water stress	4	3	1
LRR receptor-like Serine/threonine-proteinkinase	Defense against drought and salt stress	74	41	33
ERF	Enhancement of disease resistance of plants	21	10	11
GTP-binding protein	Enhancement of stress resistance and disease resistance of plants	19	6	13
Receptor protein kinase	Defense against drought and salt marsh stress	8	5	3
Disease resistance protein	Enhancement of disease resistance	56	36	20
Pathogenesis related protein	Defense against various pathogenic bacteria	13	7	6
Chitinase	Enhancement of disease resistance of plants	8	6	2
Stress protein	Enhancement of resistance of plants to external stress	14	7	7
Total		496	301	195

The differential expression of protein degradation related genes

Protein degradation which was also an important reason for plant senescence usually occurred due to the function of some proteinase and F-box family protein genes. In this study, 72 functional genes related to protein degradation were found in ancient *Camellia oleifera* leaves (Table 6). In detail, those included 17 aspartyl protease genes, 11 cysteine proteinase genes and 44 F-box family protein genes. Among cysteine proteinase, 7 genes were up regulated and 4 were down regulated. Among F-box family protein genes and aspartyl protease genes, the expression of most differential genes demonstrated a down regulated trend. With the combined effects, protein degradation of ancient *Camellia oleifera* leaves may be effectively delayed, which further was possibly helpful for the prolongation of senescence.

Table 6 Differential expression of protein degradation related genes

Gene categories	Main functions	Differential gene amount	Up regulated gene amount(Ancient tree VS Mature tree)	Down regulated gene amount(Ancient tree VS Mature tree)
F-box	Involvement in protein degradation of plant cells	44	17	27
Aspartyl protease	Involvement in senescence and pathogen related protein degradation	17	6	11
Cysteine proteinase	Involvement in protein hydrolysis	11	7	4
Total		72	30	42

Verification of transcriptome differential genes

Real time quantitative PCR was used to further verify the reliability of transcriptome sequencing results and the expression patterns of differential genes. The selected verification genes include NAC, F-box, aspartyl protease, cysteine proteinase, chlorophyll a / B and others. ETIF3H was selected as the internal reference gene. The results showed that the expression of c184012_g3 (F-box) c178940_g2 (Aspartyl protease) c163401_g1 (Cysteine proteinase) were down regulated in ancient trees, up regulated in mature tree, c167863_g1 (NAC) c184170_g1 (Chlorophyll a/b) were up regulated in ancient trees and down regulated in mature tree (Figure 2). In general, the expression patterns of these genes are consistent with the results of transcriptome sequencing, which further verifies the reliability of transcriptome sequencing results.

Discussion

The Chlorophyll content of *Camellia oleifera* leaves in ancient trees was significantly higher than those in mature tree. 9 chlorophyll a/b binding proteins in ancient *Camellia oleifera* leaves demonstrated an up regulated trend. Chlorophyll guaranteed high efficient photosynthesis of plants. Thus, the degradation of chlorophyll was also a main marker of senescence of plant leaves. In *Zea mays*¹¹, *Sorghum bicolor*¹², and *Arabidopsis thaliana*¹³, chlorophyll contents were closely related to leaf senescence. Besides, during leaf senescence, some genes involved in photosynthesis would be down regulated expressed, resulting in the decrease of the photosynthetic ability of leaves^{14,15}. Chlorophyll binding protein genes were in a down regulated trend during the senescence process of *Oryza Sativa*¹⁶ and *Gossypium*¹⁷. In this study, the chlorophyll content and the expression of chlorophyll a/b binding protein gene of ancient *Camellia oleifera* leaves demonstrated an increasing trend, illustrating that ancient *Camellia oleifera* leaves still had a high level of chlorophyll, which was very important for the maintenance of the photosynthesis of the whole tree, the promotion of the growth and development of tree and the prolongation of senescence.

Antioxidase activities of SOD, POD and CAT as well as the expression of POD-APX antioxidant related genes in ancient *Camellia oleifera* leaves demonstrated an increasing trend. In the senescence process of *Triticum aestivum*¹⁸ and *Oryza sativa*¹⁹, activities of SOD, CAT and APX decreased, which will lead to the increase of ROS level and accelerate leaf senescence. A certain concentration of ROS was necessary for the normal physiological process of plants, whereas the excessive ROS would influence and accelerate the senescence process of plants. Thus it was very important to maintain the expression equilibrium of ROS in plants for not only the growth and development but also the prolongation of senescence. Antioxidase and related functional gene expression could effectively clear ROS generated during senescence in leaves^{18,20}. In this study, multiple antioxidant activities as well as the expression of antioxidant related gene increased in ancient *Camellia oleifera* leaves. Surplus ROS in ancient *Camellia oleifera* leaves could be cleared in time, which could decrease the cell harm of ancient *Camellia oleifera* tree from surplus ROS, further maintain normal physiological metabolism of *Camellia oleifera* and prolong the senescence of plants.

Hormones were very important for the regulation of the growth and development of plants as well as the senescence process of leaves. ABA gene was up regulated expressed in the senescence process of *Gossypium*^{17,21}, which could influence the senescence of plants by the regulation of growth stress and the induction of ethylene expression^{22,23}. Abscisic acid 8'-hydroxylase which was a key functional enzyme, usually was negatively correlated with ABA content in plants²⁴. The increase of CTK level in plants can further prolong and inhibit leaf senescence by ways of decreasing Rubisco content as well as enhancing heat resistance, drought resistance, antioxidation ability and photosynthetic ability of plants^{24,25,26,27,28}. In this study, the expression of 1 CKX related gene increased in ancient *Camellia oleifera* leaves. In *Nicotiana tabacum*²⁹, the increase of the expression of AtCKX could effectively enhance the antioxidation ability of plants. Auxin related genes could prolong plant senescence by inducing auxin contents in plants and adjusting the NAC transcription factors amount^{30,31}. In *Gossypium*, the increase of the expression of auxin related gene could inhibit leaf senescence^{17,32}. In this study, the expression of 1 SAUR related gene increased in ancient *Camellia oleifera* leaves. SAUR gene played an important role in the maintenance of auxin level, the regulation of auxin transport and cell amplification^{33,34}. With the combined effects of those hormone genes, hormone levels in *Camellia oleifera* leaves could be maintained in balance, which could promote the benign growth and prolong senescence.

External growth stresses of high temperature, low temperature, drought, diseases and pests were usually closely related to plant senescence³⁵. Due to many external growth stresses, the transcription factors related to stress in plants would be induced to be expressed. The stress transcription factors could regulate the expression of the stress resistance gene in plants, thus enhancing the stress resistance of plants. In *Arabidopsis thaliana*³⁶, *Gossypium*¹⁷, *Oryza sativa*³⁷, the increase of the expression of the NAC transcription factor was very useful for prolonging plant senescence, and answering drought as well as salt and marsh stress. The MYB transcription factor expression could enhance the resistance to growth stress of drought, salt, coldness and high temperature in *Lycopersicon esculentum*³⁸, *Arabidopsis thaliana*^{39,40,41} and *Salicornia brachiata*⁴². In *Oryza sativa*⁴³ and *Arabidopsis thaliana*⁴⁴, it has been found that WRKY could influence leaf senescence by enhancing the resistance to the adverse factors of growth including drought, high temperature, pathogenic bacteria etc.

In *Trifoliate Orange*, the increase of the expression of the bHLH transcription factor could enhance POD activity of plants, clear ROS, and further enhance coldness resistance of plants⁴⁵. In *Brassica rapa*⁴⁶ and *Lycopersicon esculentum*⁴⁷, it has been found that the increase of the expression of MADS-box gene could enhance the resistance of plants to drought and salt stress. The excessive expression of the HSPs transcription factor was helpful for the enhancement of the stress resistance of plants to high temperature and drought^{48,49,50}. In *Arabidopsis thaliana*⁵¹ and *Populus Trichocarpa*⁵², it has been found that the increase of the expression of the functional gene of Zinc Finger protein could enhance the stress resistance of plants to drought, high temperature, salt stress and pathogen by the adjustment of antioxidase activities of plants^{53,54}. Zhu *et al.* found that the slbzip1 transcription factor of Leucine zipper could play an important role in the resistance of *Lycopersicon esculentum* to salt and drought by regulating the pathway mediated by ABA⁵⁵.

Similar with the transcription factor, plant received stress stimulation, led to the physiological change of plant cells through many signal transduction pathways and finally adapted to the change of external environment^{56,57}. GTP binding protein was very important for the protection of cell signal transduction and the enhancement of the stress resistance and the disease resistance of plants^{58,59}. It showed that receptor protein kinase played an important role in growth stress resistance to drought and salt marsh as well as stress responses of antioxidation defense and pathogen reaction^{57,60,61}. LRR receptor-like serine/threonine protein kinase (FLS2) usually played an important role in the interaction pathway of plant-pathogen, involved in the defense system of pathogen and finally greatly enhanced the disease resistance ability of plants⁶². Ethylene-responsive transcription factors (ERF) not only involved in the plant senescence and the regulation of many stress response but also took part in the defense and stress answering reaction of plants^{63,64,65}.

In the growth process, plant would not only be influenced by external growth stress but also encountered the invasion of many pathogenic bacteria, which finally exacerbated the senescence process. Chitinase was verified to effectively inhibit activities of some pathogens, intensify the defense system of plants and enhance the disease resistance of plants to many pathogenic bacteria^{66,67}. when encountering stress, plant cells could also increase the defense gene expression of resistance protein, defense-related gene etc. to prevent cells from harm. In ancient trees, the whole gene expression of disease resistance protein and pathogenesis related protein demonstrated an increasing trend.

In this study, the expression of multiple signal transduction factors, transcription factors and disease resistance related genes were up regulated. With the combined effect of those functional genes, *Camellia oleifera* could

response to external stress and some pathogen invasion in time, enhance resistance to various growth stresses and pathogenic bacteria through those factors and finally prolong senescence.

There were many differential genes related to stress resistance of *Camellia oleifera*, which was related to the growing environment of *Camellia oleifera* itself. *Camellia oleifera* was a specific economic tree species in China, which mainly distributed at low mountains and hills in different provinces of South China. In those areas, during the growth process, *Camellia oleifera* encountered stresses of low temperature in winter, lots of rain in spring, high temperature in summer and drought. Besides, *Camellia oleifera* was susceptible to anthracnose and soft rot. By preliminary speculation, due to the influence of those growth stresses and pathogenic bacteria, the expression of many resistance related genes in *Camellia oleifera* including the transcription factors, the resistance factors, the defense factors and the signal transduction genes, which could better defend against those adverse growth factors and finally promote normal growth and development of plants.

In ancient *Camellia oleifera* leaves, the overall expression of functional genes related to protein degradation were down regulated. Similar with the situation of chlorophyll degradation, protein would also be degraded during plant senescence¹⁵. Cysteine proteinase was regarded as the most abundant enzyme which was related to plant leaf senescence⁶⁸. and in protein hydrolysis⁶⁹. The expression of aspartic protease which took part in petal senescence as well as pathogen related protein degradation⁶⁹ would be increased during senescence in *Glycine max*⁶⁹ and *Arabidopsis thaliana*⁷⁰. F-box gene was one of the largest gene families which took part in cell protein degradation in plants. Encountering stresses and during leaf senescence, F-box gene was up regulated expressed in chickpea⁷¹ and *Oryza sativa*⁷². With the degradation of protein, the senescence process of plants would be exacerbated. In ancient *Camellia oleifera* leaves, the expression of aspartyl protease gene, cysteine proteinase gene and F-box family protein gene were decreased, which effectively inhibited the degradation of protein and thus prolonged senescence.

Conclusions

This study shows that: (1) With the increase of the tree age of *Camellia oleifera*, the chlorophyll content in ancient *Camellia oleifera* leaves was significantly higher than those in mature and young trees, which was also verified by the chlorophyll related gene expression in *Camellia oleifera* leaves. Multiple chlorophyll a/b-binding proteins in ancient *Camellia oleifera* leaves demonstrated an up regulated trend, illustrating more active vital movement in ancient *Camellia oleifera* leaves.

(2) The whole expression of related genes of POD, CAT and APX in ancient *Camellia oleifera* leaves demonstrated an up regulated trend, which was verified by antioxidase contents of SOD, POD and CAT in ancient *Camellia oleifera* leaves, illustrating that the clearance ability of ROS in ancient *Camellia oleifera* leaves could be enhanced by the increase of antioxidase activities.

(3) The expression of multiple antisenesescence hormone related genes increased in *Camellia oleifera* leaves, wherein auxin related gene number was the most.

(4) Multiple stress resistance and pathogenic bacteria resistance related genes were found in ancient *Camellia oleifera* leaves. With the combined effects of those genes, *Camellia oleifera* could answer various external

growth stress and pathogenic bacteria in time and further response correspondingly by the corresponding transcription factor, signal transduction, resistance protein and defense related gene.

(5) The whole expression of the functional differential genes of Aspartyl protease, Cysteine proteinase and F-box family protein, which influenced protein degradation, demonstrated a decreasing trend in ancient *Camellia oleifera* leaves. Thus, protein degradation would be effectively inhibited.

(6) The results of real-time quantitative PCR were consistent with those of transcriptome sequencing, which further verified the reliability of transcriptome sequencing results.

(7) With the comprehensive effects of those factors, ancient *Camellia oleifera* leaves still kept an exuberant vitality, which had a high reference value for the stress resistance biological study and genetic improvement research of *Camellia oleifera*.

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Figures



Figure 1

The growth status of *Camellia oleifera* trees with different ages Note: Those from left to right were ancient *Camellia oleifera* (> 100 a), mature tree (30 a).



Figure 2

RT-qPCR was used to detect the expression of five differential genes Note: P1 is ancient *Camellia oleifera* (> 100 a), P2 is mature tree (30 a).

Supplementary Files

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- [SupplementaryTable1Differentialgenestatics.xls](#)
- [SupplementaryTable2Differentialgeneannotation.xlsx](#)